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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/057,505	01/25/2002	Roger Y. Tsien	REGEN1260-3	7832

7590 06/02/2004

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EXAMINER	
ROBINSON, HOPE A	

ART UNIT	PAPER NUMBER
1653	

DATE MAILED: 06/02/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/057,505	TSIEN ET AL.	
	Examiner	Art Unit	
	Hope A. Robinson	1653	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *15 March 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *57-78* is/are pending in the application.

4a) Of the above claim(s) *62-75,77 and 78* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) *57-61 and 76* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☒ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's election of Group I (57-61 and 76) with traverse on March 15, 2004 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Claim Disposition

2. Claims 1-56 have been canceled on January 25, 2002. Claims 57-78 have been added. Claims 57-78 are pending. Claims 57-61 and 76 are under examination.

Oath/Declaration

3. The Oath/Declaration is objected to because non-initialed and/or non-dated alterations have been made to the oath or declaration. See 37 CFR 1.52(c). See for example where corrections have been made to citizenship information.

Drawing

4. The drawings filed on January 25, 2002 have been received and entered.

Specification

5. The specification is objected to because of the following informalities:

The specification is objected to because on page 16 Table I appears with a black background. The table should be replaced with a table with a white background to ensure that it reproduces well when printed. In addition, the status of U.S. application listed throughout the specification should be updated see for example page 35, line 3. Correction is required.

Claim Objections

6. Claims 57, 58 and 76 are objected to because the claims do not have the proper sequence notation, as the claims recite "(SEQ.ID.No.2)" instead of "(SEQ ID NO:2)".

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

7. Claims 57-61 and 76 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are directed to a tandem fluorescent protein construct, comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit FRET when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties (see for example claim 57 or 76). The claims are also directed to donor and acceptor moieties comprising SEQ ID NO:2 comprising several amino acid substitutions that are combined, however, the open language "comprising" encompasses other mutations not defined which could result in a structure that would not produce FRET or a substantially different protein than SEQ ID NO:2 with the mutations disclosed or a protein that is not biologically functional. Thus, the claims encompass a genus of proteins not described or defined. The claims recite the open language "comprising" therefore, the claims encompass any structure as long as said structure comprises the above mutation. Additionally, the specification fails to describe or provide any identifying characteristics or properties or provide data to demonstrate that function is retained in the "other" mutations. Further the specification lacks adequate written description with the respect to the linker as page 8 defines the linker as a "radical", the same definition is applied to the fluorescent protein moieties which is problematic with respect to the energy transfer desired. Therefore, for all these reasons the specification lacks adequate written description, and one of skill in the art cannot reasonably conclude that the applicant had possession of the claimed invention at the time the instant application was filed.

8. Claim 57-61 and 76 are rejected under 35 U.S.C. 112, first paragraph, because

the specification, while being enabling for a donor fluorescent protein moiety and an acceptor fluorescent moiety contained in SEQ ID NO:2 with specific mutations to SEQ ID NO:2 at the positions listed in for example claim 57, producing a structure that is substantially homologous to SEQ ID NO:2 and the disclosure in U.S. Patent No. 5,981,200, (for example, wherein the linker is a peptide moiety that does not emit light to excite the donor fluorescent protein moiety), does not reasonably provide enablement for mutations to the donor and acceptor moieties "comprising" (claims 57, 58 and 76) other mutations that are encompassed by the open language which could result in a structure that would not produce FRET or a substantially different protein than SEQ ID NO:2 with the mutations disclosed or a protein that is not biologically functional. The specification is also not enabled for any linker as the linker moiety may refer to a single amino acid or a group or any linker with a protease recognition site for any protease (see claims 57, 58 or 76). While the specification is enabled for linkers that are not fluorescent, is not enabled for linkers that are fluorescent. Further, the specification while enabled for linkers about 5-50 amino acids (see page 2 of the specification) is not enabled for linkers with the lengths encompassed by the claims.

The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to practice the invention commensurate in scope with these claims. There are many factors to be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is undue. These factors include, but are not limited to: quantity of

experimentation necessary; amount of direction or guidance presented; presence or absence of working examples; nature of the invention; state of the prior art relative skill of those in the art; predictability or unpredictability of the art and breadth of the claims, each of which will be discussed below.

The claims are directed to a tandem fluorescent protein construct, comprising a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties and wherein the donor and acceptor moieties exhibit FRET when the donor moiety is excited by radiation, characterized in that the linker moiety comprises a protease cleavage recognition site, wherein cleavage of the linker by a protease results in a change in FRET between the donor and acceptor moieties (see for example claim 57 or 76). The specification on page 8, line 12-15 appears to describe linkers as encompassing in scope those molecules that can be fluorescent in the same manner as the donor and acceptor moieties (in defining the "linker moiety" as a "radical" in the same manner as the fluorescent protein moieties). The specification only provides guidance for the use of linkers as a non-fluorescent moiety that provides at least the appropriate degree of separation between donor and acceptor moieties. There is no guidance to use the linker in any other manner, and the effect of having an additional fluorescent moiety between the donor and acceptor would have unpredictable consequences on resonance transfer, which as taught on page 12 of the instant specification is extremely sensitive to the degree of separation between donor and acceptor. One of skill in the art would have to engage in undue experimentation to provide linkers with the

properties encompassed by the claims given these factors. The claims are also directed to donor and acceptor moieties comprising SEQ ID NO:2 comprising several amino acid substitutions that are combined. The open language of comprising in the claims signifies that mutations beyond those listed can occur in the sequence which might not be tolerated. Therefore the claims encompass undefined structures or multiple fluorescent moieties for which the specification does not provide enablement.

Additionally, the specification fails to describe or provide any identifying characteristics or properties for the "other mutations" encompassed in the open claim language or provide data to demonstrate that function is retained or that the protein moieties exhibit FRET. Therefore, while it is known that many amino acid substitutions are generally possible in any given protein the positions within the protein's sequence where such amino acid substitutions can be made with a reasonable expectation of success are limited, as certain positions in the sequence are critical to the protein's structure/function relationship. For example, Heim et al. (PNAS, vol. 91, pages 12501-04, 1994) disclose that a mutated DNA was sequenced and found to contain five amino acid substitutions, only one of which was found to be critical, Tyr66His, in the center of the chromophore. Heim et al. also disclose further site directed mutagenesis and noted that there was tolerance of the substitutions made, however, some mutants were weakly fluorescent (page 12504). The substitutions contemplated by the instant invention is greater than that proposed in the art, hence the specification should provide guidance as to what portion of the sequence is conserved and define the "other mutations" encompassed in the "comprising" language.

In addition, the specification on page 20, line 31 discloses that the optimal distance between the donor and acceptor sites is between about 1nm to about 10nm for the claimed resonance energy transfer to be useful. However, the "fluorescent protein moieties" encompass fluorescent peptide fragments of the intact fluorescent proteins, the distance between donor and acceptor may be about as short as the length of the linker. On page 20 of the specification it is stated that the length of the linker moiety is chosen to optimize both FRET and the kinetics and specificity of enzymatic cleavage. Thus, if the linker is too short, the protein moieties may sterically interfere with each other's folding or with the ability of the cleavage enzyme to attack the linker. However, the claims broadly encompass linkers that are greater than 5-50 amino acids or 1-10nm in length which is not supported by the instant specification that discloses that linker length is a critical parameter required for the tandem conjugates to work and that linker lengths beyond about 1-10nm would unpredictably result in interference with polypeptide folding, enzyme cleavage, insufficient resonance transfer, or linker cleavage specificity. Moreover, the claims recite two fluorescent protein moieties said to be linked to one another via a linker moiety, the specification does not provide guidance as to covalent binding occurring via cyclization and oxidation of amino acids of the donor and acceptor protein moieties, or via any other methods considered to produce the "coupling" of the donor and acceptor protein moieties. No information is provided as to how the individual fluorescent moieties are to be isolated and ultimately linked to one another via any linking moiety. Thus, absent adequate guidance/direction regarding for example, the linker length, based on the breath of the claims, the undefined structures

encompassed by the claims, the nature of the invention and the unpredictability of the linker as recited in the claims, a skilled artisan would not be able to practice the claimed invention commensurate in scope with the claims.

In view of the foregoing, one of skill in the art would require guidance, beyond that provided in the instant specification, in order to make the claimed tandem fluorescent protein in a manner that reasonably correlated with the scope of the claims. Without such guidance, the experimentation left to those skilled in the art is undue.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

9. Claims 57-61 and 76 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 57, 58 and 76 lack antecedent basis for "the donor and acceptor moieties", it is suggested that the claims are amended to recite "said donor fluorescent moiety and said acceptor fluorescent moiety". The claims are also indefinite because it is unclear if the donor fluorescent protein moiety emits light as a result of excitation from radiation only or if the donor fluorescent protein moiety emits light as a result also of the linker emitting light to excite the donor fluorescent protein moiety (see also the disclosure in the specification on page 8 as to the definition of the linker). The claims

are also indefinite for the recitation of improper Markush language with respect to the listing of the substitutions. It is suggested the claims are amended to recite "comprising the amino acid substitutions selected from the group consisting of (a); (b); (c); (d); (e); or (f); ...." (see also the language for the acceptor moiety). In addition the phrase "comprising the amino acid substitutions," should be amended to "comprising the amino acid substitutions:", for clarity. The dependent claims are also included in this rejection.

Claim 59 is indefinite because the claim has improper multiple dependent language, it is suggested that the claim is rewritten as "The construct of any one of claims 57 or 58" (see also claim 60).

The Basis For Non-Statutory Double Patenting

10. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double

patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

11. Claims 57-61 and 76 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-4, 7-12 and 14 of U.S. Patent No. 5,981,200. Although the conflicting claims are not identical, they are not patentably distinct from each other because the claims in each are directed to tandem fluorescent protein constructs comprising a donor fluorescent moiety, an acceptor fluorescent moiety linked by a linker moiety, wherein the donor and acceptor moieties exhibit fluorescence resonance energy transfer (FRET) when said donor is excited and wherein the linker moiety has a protease cleavage recognition site. Both sets of claims recite substitutions that can occur to the donor and acceptor moieties which comprise an *Aequorea* fluorescent protein. Note that the modifications contemplated in the patent are encompassed in the instant application and therefore the limitations in the instant application are considered obvious in light of the patented claims; the claims of the patent are generic to the instant claims. Therefore, the claims of the patent and the instant application claims are an obvious variation of each other.

Conclusion

12. No claims are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Hope A. Robinson whose telephone number is 571-272-0957. The examiner can normally be reached on Monday-Friday from 9:00 a.m. to 6:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher S.F. Low can be reached on 571-272-0951. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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Patent Examiner